Calif.-28

VARIATIONS IN COLLETOTRICHUM GLOEOSPORIOIDES

BY

O. F. BURGER

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XX, No. 9 : : : : Washington, D. C., February 1, 1921

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1921

VARIATIONS IN COLLETOTRICHUM GLOEOSPORIOIDES[1]

By O. F. Burger[2]

Instructor in Plant Pathology, Graduate School of Tropical Agriculture and Citrus Experiment Station, University of California

The diseases of citrus trees and fruit known as wither-tip, leafspot, anthracnose, and tearstain are all caused by the same fungus, *Colletotrichum gloeosporioides* (Penz.). These diseases have been found in Florida, (*4; 5; 9, p. 88*),[3] California (*3*), West Indies, South America, Australia, and Malta; and in practically all citrus-growing regions rather serious outbreaks of some or all of these diseases have occurred from time to time.

The smaller twigs of citrus trees are very frequently and severely attacked by the fungus. It is quite common to see many of the small twigs killed back 4 or 5 inches. These infected twigs soon turn to a light brown color and sooner or later become dotted over with numerous small black acervuli. After the rainy season begins, the spores, which are imbedded in a gelatinous matrix, exude from the acervuli and are washed down over the fruit and leaves, causing leafspot, tearstain, and anthracnose of the fruit.

The spores must have an abundance of moisture in order to germinate. Since the rainy season in California occurs during the winter and early spring months, it is at this period that these diseases are most prevalent. In Florida these diseases cause much damage to the citrus industry, whereas in California they are considered of minor importance. This difference in the amount of injury in the two States named is due, I believe, to the difference in the amount of rainfall. During the dry summer in California there is little evidence that *Colletotrichum gloeosporioides* is active. In Florida this fungus causes bloom drop and a considerable amount of leaf spotting during the spring and summer months, as well as anthracnose and tearstaining of the ripe fruit. Many growers and agricultural workers believe that the fungus injury is secondary. It has been stated repeatedly that the weak or injured tree is more susceptible to an attack of *C. gloeosporioides* than the healthy tree.

DESCRIPTION AND HISTORY OF THE FUNGUS

The fungus, *Colletotrichum gloeosporioides* (Penz.) was first described by Penzig in 1882 as *Vermicularia gloeosporioides*. In 1887 he placed

[1] Paper No. 66, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Resigned June 1, 1918.

[3] Reference is made by number (italic) to "Literature cited," p. 735-736.

Journal of Agricultural Research,
Washington, D. C.
ws

Vol. XX, No. 9
Feb. 1, 1921
Key No. Calif.-28

it in the genus Colletotrichum. It was first collected in America in 1886 by Dr. Martin from Green Cove Springs, Fla., and was first reported by L. M. Underwood (*8*) in 1891. However, the disease was not found in California until some years later. It was reported by Essig (*4*) in 1909 from the Limoneira Ranch at Santa Paula, where it was causing considerable damage to lemon trees.

In 1904, Prof. P. H. Rolfs (*5*) gave a very good description of the fungus as it occurred on various citrus trees and fruits in Florida. He says (*5, p. 20*) that the—

> diseases . . . manifest themselves as wither-tip on orange, pomelo, and lemon twigs; as leaf-spot on the leaves of the various citrous species; as anthracnose on lime blossoms, recently set limes, lime twigs, and lemon twigs; as lemon-spot on ripe lemons and as canker of limes.

The following description is given by Prof. P. H. Rolfs:

> Acervuli located on the surface of the leaf, twig or fruit; 90–270 μ in diameter, erumpent, superficial. Shape various, not uniform, occurring on either surface of citrus leaves; disposed irregularly or in more or less concentric lines; pale to dark colored. On tender lime twigs, tender lemon twigs, lemon fruits and lime fruits, pale colored, dull red in masses, confluent. Epidermis breaks irregularly. Setae fuliginous, ranging in length from 60–160 μ, frequently once or twice septate, disposed at margin of acervuli. Frequently absent, and on tender lime twigs, tender lemon twigs, lemon fruits and lime fruits usually absent.
>
> Conidia broadly oval or oblong, 10–16 μ by 5–7 μ, hyaline; size variable in same acervulus, usually with one or two oil drops. Developing from a well-defined stroma; basidia, 3–18 μ. In moist chambers the conidia stream from the break in the epidermis. Intrabasidial setae, variable 8–30μ by 3–6μ, cylindrical or sometimes enlarged at distal end; hyaline.

In 1912 Clausen (*1*) described the fungus causing wither-tip of the lime, *Citrus medica*, as *Gloeosporium limetticolum*. He believes that Rolfs had confused two forms and described them as one. Clausen uses the absence of setae as a distinguishing character from *Colletotrichum gloeosporioides*. It is the opinion of Stoneman (*7*), Edgerton (*2*), and Shear and Wood (*6*) that the setae are variable as to presence or absence and that they are not reliable morphological characters to use in separating genera. I have found them in some of my cultures of *Colletotrichum gloeosporioides*, while in other cultures they were absent. Another character he uses is the lack of a coarsely granular plasma filling the spores. I have found several strains of this fungus which are considered to be *Colletotrichum gloeosporioides*, whose spores are not filled with a coarse granular plasma but appear at first to be homogeneous. Clausen also uses growth characteristics as a means to identify the two strains. Some of my strains had the same growth characteristics as the strain which was obtained from Clausen—that is, a white mycelium and abundant spore production.

Shear and Wood (*6*) in their bulletin on the genus Glomerella, have brought together strains from various hosts and included them in one

species, *Glomerella cingulata*. To my knowledge, the perfect stage of Clausen's fungus has not been found. Several of my strains produced the perfect stage when first isolated, and the spores and asci were the same as described for *G. cingulata*. It is, therefore, the opinion of the writer, which will be presented in the following pages, that *Colletotrichum gloeosporioides* as found in California is a polymorphic species, composed of many strains.

STRAINS IN COLLETOTRICHUM GLOEOSPORIOIDES

In the fall of 1916 when the writer began work at the Citrus Experiment Station, the wish was expressed that he should study *Colletotrichum gloeosporioides*. The different members of the Division of Plant Pathology had isolated several cultures of this fungus from different citrus hosts. Some of these differed from each other in their cultural characteristics. It was suggested that these forms might have different regional distribution, or that their differences might be due to the host. Other isolations were made from the various citrus hosts; and these, together with the cultures obtained from the different members of the Division of Plant Pathology, were given laboratory numbers and were always spoken of as strains. In all, 46 cultures were used in the study. Forty-two of these represented all the important citrus districts of southern California, and there was one each from Texas, Florida, Alabama and one kindly furnished by Dr. C. L. Shear.

CULTURAL CHARACTERISTICS

The various strains were grown on five different media—corn meal agar, green bean plugs, potato agar, lactose-beef agar and oatmeal agar. Each strain was grown on these five different media for a period of 18 months. Transfers were made about every 5 weeks, and a record was kept of the variations in growth occurring in each strain on the various media. While most of the strains exhibited different cultural characteristics on the various media, there were a few whose macroscopic characteristics of the mycelium were much the same on all the media. Not only did each strain vary in its growth characters on the different media but some of the strains differed characteristically from each other. Therefore, the variations exhibited by the various strains in their cultural characteristics made it possible to classify them into the following five groups.

Group I: Mycelium white; spores abundant, salmon-colored in mass.

Group II: Mycelium grey to greenish black on the various media, very little aerial growth on oat agar; spores abundant, salmon-colored or yellowish in mass.

Group III: Mycelium gray to black on various media; no spore masses on oat agar.

Group IV: Mycelium gray to black; spore production so abundant on all media that the surface of the medium is nearly covered by a bacteria-like mass of spores.

Group V: Mycelium gray to black, rather fluffy; no pink spore masses on any medium; spore production scant and on some media no spores produced.

Since the cultural characteristics of some strains changed, it became necessary to reclassify the different strains on the following dates: January 27, 1917; April 16, 1917; September 13, 1917; and February 28, 1918. Very few of the strains remained in the group in which they were placed at the first classification. Under artificial cultivation the characteristics of the various strains changed; therefore, they were placed in different groups (see Table I). There were only three strains whose characteristics remained constant in group I. In group II there was only one strain which remained constant. It will be noticed that in group IV cultures 296 and 299 remained constant until September 13, 1917. At the next date of classification these two strains were placed in group II. No strains were placed in group V until September 13, 1917. This may be due to the fact that under artificial conditions these strains lost their power to produce spores.

TABLE I.—*Classification of strains of Colletotrichum gloeosporioides into groups*

Group No.	Jan. 22, 1917.			Apr. 16, 1917.			Sept. 13, 1917.			Feb. 28, 1918.		
I	[a] 295			[a] 295			[a] 295	[a] 429	934	[a] 295		
	[a] 298			[a] 298			[a] 298	496	955	[a] 298		
	323			323			323	502		[a] 429		
	[a] 429			[a] 429			326	901		496		
										561C		
II	326	475	[a] 990	326	560	[a] 990	325	[a] 990		296	527	926
	459	483		459	561		475			299	536	[a] 990
	496	651		475	651		483			323	536A	
	502	901		483	912		510			325	536B	
	507	912		502	926		536			326	561B	
	510	926		507	934		612			502	620	
	943	940		536	943		615			510	901	
III	297	934		297	496	955	507	912		406	536C	912
	325	955		325	510		560	943		475	560	943
	406			406	910		561			507	561	955
	467			467	940		651			527A	561A	
											527C	612
IV	296			296			296					
	299			299			299					
V							297			297	934	
							467			483	940	
							495			495		
							527			527B		
							940			651		

[a] Culture remained in its original class throughout the work.

VARIATIONS IN SPORE LENGTH

Since such great differences were found in cultural characteristics between the strains, the question arose whether differences could be found in the spore length of the various strains. One hundred spores were measured from each strain. The measurements were made in the fol-

lowing manner: A dilute suspension of the spores taken from green bean plugs was made in sterilized tap water, and a drop of the suspension was placed on a microscope slide and covered with a cover glass. It was necessary to make the measurements quickly, because the spores did not remain quiet for any length of time. The image of the spore was thrown on drawing paper by means of the camera lucida, and the length and width were quickly marked with a pencil. The microscope was so adjusted that 1 micron on the micrometer scale in the eyepiece was equal to 1 millimeter on the paper. Therefore, after the length and width were indicated on the paper the spore size could be quickly ascertained by means of a millimeter rule.

TABLE II.—*Variation in spore length in the different strains of Colletotrichum gloeosporioides*

Strain No.	Number of spores measuring (in microns)—																			
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
296	1	1	3	9	19	28	18	14	6	....	....	....	....	....	....	....	....	....	....	....
901	....	....	....	....	....	....	....	....	1	1	7	10	12	21	19	16	10	1	1	1
459	....	....	....	....	....	4	5	26	33	27	4	1	....	....	....	....	....	....	....	....
429	....	....	....	1	1	2	10	29	31	18	5	2	0	1	....	....	....	....	....	....
406	....	....	....	1	0	2	4	4	13	21	27	11	12	1	2	2	....	....	....	....
326	....	....	....	....	....	....	3	4	15	25	28	15	8	0	1	....	....	....	....	....
955	....	....	....	1	5	23	17	29	21	2	0	1	....	....	....	....	....	....	....	....
295	....	....	....	1	4	14	16	20	28	8	7	2	....	....	....	....	....	....	....	....
990	1	0	0	0	1	1	2	8	35	25	19	5	3	....	....	....	....	....	....	....
297	....	....	....	....	....	4	4	25	27	23	14	1	2	....	....	....	....	....	....	....
651	....	....	....	....	1	1	0	1	8	16	33	21	12	4	3	....	....	....	....	....
299	....	....	....	....	....	15	16	20	32	13	3	0	1	....	....	....	....	....	....	....
323	....	....	....	....	6	24	33	23	11	1	2	....	....	....	....	....	....	....	....	....
510	....	....	....	....	....	3	4	15	41	19	9	7	1	1	....	....	....	....	....	....
507	....	....	....	....	....	7	22	27	37	6	1	....	....	....	....	....	....	....	....	....
502	....	....	....	....	....	13	32	38	13	2	1	0	1	....	....	....	....	....	....	....
943	....	....	....	....	....	4	6	17	26	18	16	11	2	....	....	....	....	....	....	....
940	....	....	....	....	....	....	6	25	37	21	8	2	1	....	....	....	....	....	....	....
934	....	....	....	2	0	5	10	18	32	16	16	0	1	....	....	....	....	....	....	....
527	....	....	5	16	21	22	11	8	5	3	5	4	....	....	....	....	....	....	....	....
560	....	....	....	....	....	11	16	23	28	13	2	3	3	0	1	....	....	....	....	....
536	....	....	....	....	....	1	2	9	21	30	20	12	4	0	1	....	....	....	....	....
561	....	....	....	....	....	2	3	12	36	20	20	4	1	2	....	....	....	....	....	....
524	....	....	....	....	....	....	2	5	22	39	20	10	2	....	....	....	....	....	....	....
514	....	....	....	....	....	2	1	9	20	33	19	11	5	....	....	....	....	....	....	....
513	....	....	....	....	....	....	1	8	24	43	13	8	3	....	....	....	....	....	....	....
517	....	....	....	....	....	1	1	8	19	44	18	8	1	....	....	....	....	....	....	....
515	....	....	....	....	....	....	1	2	8	21	28	22	13	3	2	....	....	....	....	....
947	....	....	....	....	....	....	2	3	19	35	27	12	0	0	2	....	....	....	....	....
467	....	....	....	....	....	1	2	6	22	21	25	13	9	1	....	....	....	....	....	....
512	....	....	....	....	....	....	4	4	20	36	27	5	2	1	0	1	....	....	....	....
475	....	....	....	....	....	1	2	7	22	26	26	12	2	1	1	....	....	....	....	....
926	....	....	....	....	....	....	3	16	31	27	17	5	1	....	....	....	....	....	....	....
483	....	....	....	....	....	2	2	8	26	28	21	10	1	1	0	1	....	....	....	....
325	....	....	1	0	0	7	11	39	30	9	2	0	0	1	....	....	....	....	....	....
298	....	....	....	1	0	5	9	24	31	21	6	3	....	....	....	....	....	....	....	....

It was soon determined that each strain had a certain range of variability in its spore length and width (see Table II). While there were

individual variations exhibited, yet it was soon determined that many of the strains had the same mode for their spore lengths. Therefore, the cultures were classified in regard to the modal length of the spores (see Table III). The strains varied in their modal spore length from 12 to 20 μ. Most of the strains have their mode at 15 μ.

TABLE III.—*Mode of the spore length of different cultures of Colletotrichum gloeosporioides*

Spore No. measuring (in microns)—								
12	13	14	15	16	17	18	19	20
296	323	325	295	475	326	.....	.....	901
527	.....	502	297	483	406	.....	.....	.....
.....	.....	955	298	512	467	.....	.....	.....
.....	.....	.....	299	513	515	.....	.....	.....
.....	.....	.....	429	514	651	.....	.....	.....
.....	.....	.....	459	517	912	.....	.....	.....
.....	.....	.....	507	524	.....	.....	.....	.....
.....	.....	.....	510	536	.....	.....	.....	.....
.....	.....	.....	560	947	.....	.....	.....	.....
.....	.....	.....	561	.....	.....	.....	.....	.....
.....	.....	.....	926	.....	.....	.....	.....	.....
.....	.....	.....	934	.....	.....	.....	.....	.....
.....	.....	.....	940	.....	.....	.....	.....	.....
.....	.....	.....	943	.....	.....	.....	.....	.....
.....	.....	.....	990	.....	.....	.....	.....	.....

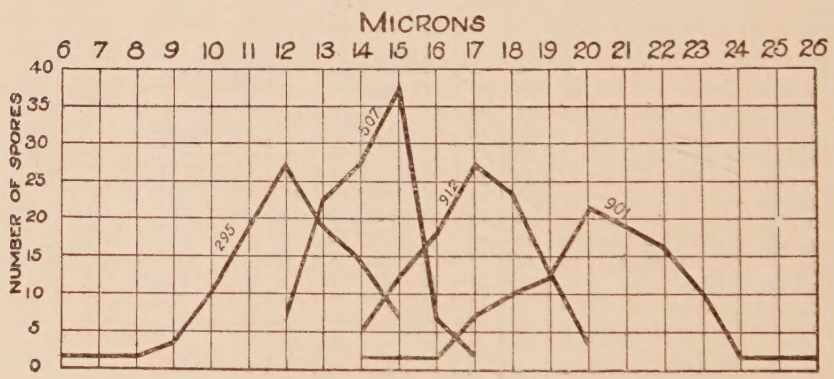


FIG. 1.—Variability of strains of *Colletotrichum gloeosporioides* in spore length.

It was soon observed that this classification could not be correlated with the classification of the strains based on their cultural characteristics. It was hoped that it would be possible to find morphological differences correlated with the cultural characters, but this was not the case.

In order to show the variability within the strain and the differences between the strains, graphs were made representing the variability in four strains (fig. 1). Strain 296 has its modal spore length at 12 μ, 507

has its mode at 15 μ, 912 has its mode at 17 μ, and strain 901, which has the largest spores of all the strains, has its mode at 20 μ.

There was also a certain range of variability in spore width. The variability was not as great as in length. The widths ranged from 3 to 8.5 μ; in most of the strains the mode was about 4 or 5 μ. In strain 901 the variability was from 5 to 8.5 μ with the mode at 6.5 μ.

In Table IV are given the calculated mean, standard deviation, and probable error of each, for the spore length and width of eight different strains. The measurements were made from spores taken from the green bean plug medium.

TABLE IV.—*Table of calculated spore measurements for certain strains of Colletotrichum gloeosporioides*

Strain No.	Mean length of spore in microns.	σ	Mean width of spore in microns.	σ
295	11.54±0.065	0.97±0.046	5.52±0.057	0.85±0.041
296	12.01± .115	1.71± .082	4.2 ± .065	.97± .046
298	14.79± .094	1.40± .067	4.68± .014	.21± .010
429	14.73± .095	1.42± .068	3.26± .077	1.15± .055
507	14.16± .079	1.17± .056	4.91± .048	.71± .034
651	17.23± .110	1.64± .078	4.52± .035	.52± .025
901	20.34± .137	2.04± .097	6.45± .132	1.96± .093
912	16.99± .097	1.44± .069	4.7 ± .110	1.63± .078

This table shows that strains grown on the same medium under like conditions vary greatly in respect to their spore sizes. We can, therefore, safely conclude that there exist individual differences in the various strains in regard to certain morphological characters.

VARIATIONS IN THE DIFFERENT STRAINS INDUCED BY THE MEDIUM

The difference in growth characteristics occurring in the same strain when transferred to the various media was very noticeable. The various strains were grown on the five different media for a period of one year. Transfers were then made from cultures growing on the various media to different plates poured with the same medium. The plates were kept at room temperature, and their growth characteristics were noted. It was soon observed that some strains had been more affected than others by their previous environment. While some of the variations were slight, still it was impossible to account for this variation other than as the effect of the medium.

On October 25, 1917, 20 cc. of potato agar were poured in sterilized Petri dishes and allowed to harden. Transfers were then made from the various strains as follows:

STRAIN 429

Plates 1 to 4 were transfers from mycelium on corn meal agar.
Plates 5 to 8 were transfers from spores on corn meal agar.
Plates 9 to 12 were transfers from mycelium on green bean plugs.
Plates 13 to 16 were transfers from mycelium on glucose-potato agar.
Plates 17 to 20 were transfers from mycelium on lactose-beef agar.
Plates 21 to 24 were transfers from mycelium on oatmeal agar (spores).
Plates 25 to 30 were transfers from mycelium on oatmeal agar (mycelium).
On November 22 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 4. White, woolly fungal growth covering the medium. Plate No. 4 was distinctly zoned; spores in center of culture.
Plates 5 to 8. White, scanty fungal growth, which gave the culture a granular appearance; spores in center of culture.
Plates 9 to 12. White, cottony growth, not zoned, but in two plates there was considerable dark mycelial growth; spores in center of culture.
Plates 13 to 16. Very scanty white mycelial growth; few spores.
Plates 17 to 20. White, cottony growth; no spores.
Plates 21 to 24. A membrane-like growth over the entire surface. Very little aerial growth; few spores.
Plates 25 to 30. White, scanty growth of a granular appearance; zoned.

STRAIN 561

Cultures made on glucose potato agar, December 18, 1917.
Plates 1 to 5 were transfers from corn meal agar.
Plates 6 to 10 were transfers from glucose-potato agar.
Plates 11 to 15 were transfers from oatmeal agar.
The final notes were taken on December 28, 1917.
Plates 1 to 5. There is a gray, woolly aerial mycelium; growth in medium is dark In plate 1 there is a white sector; no aerial growth but abundant spore production.
Plates 6 to 10. The growth is white, apprest, wet-looking; no spores.
Plates 11 to 15. No aerial mycelium, zoned, growth in medium white; good spore production on surface.

STRAIN 560

Cultures were made on Petri dishes, poured with corn meal agar December 5, 1917.
Plates 1 to 3 transferred from corn meal agar tubes.
Plates 4 to 6 transferred from green bean plug.
Plates 7 to 9 transferred from glucose-potato agar.
Plates 10 to 12 transferred from lactose-beef agar.
Plates 13 to 15 transferred from oatmeal agar.
On December 17 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 3. White growth in medium; good spore production.
Plates 7 to 9. White growth in medium; no aerial growth; no spores.
Plates 10 to 12. White, woolly aerial growth; no spores.
Plates 13 to 15. Growth in medium, dark; very scant aerial growth; no spores.

STRAIN 990

On October 16, 1917, corn meal agar plates were inoculated with strain 990, the transfers being made from the various media.
Plates 1 to 4 transferred from corn meal agar tube.
Plates 5 to 8 transferred from green bean plug.
Plates 9 to 12 transferred from glucose-potato agar tube.

Plates 13 to 16 transferred from lactose-beef agar tube.
Plates 17 to 20 transferred from lactose-beef agar tube.
Plates 21 to 24 transferred from oatmeal agar (mycelium).
Plates 25 to 28 transferred from oatmeal agar (spores).
The final notes were taken October 29, 1917.
Plates 1 to 4. Gray, short mycelial growth.
Plates 5 to 8. Gray to black aerial mycelium, but in some spots there were no aerial hyphae, growth confined to the medium; good spore production. The peculiar spots were more or less in sector-like areas. Plate 6 showed definite sectors of black and gray aerial mycelium, and in some sections the growth was confined in the medium.
Plates 9 to 12. Almost all the plates had a good growth of gray aerial mycelium, while in others there appeared sectors where the mycelium was confined in the medium.
Plates 13 to 16. No aerial mycelium, but the growth was confined in the medium, was light-colored, and was producing many spores.
Plates 17 to 20. The aerial growth is gray, woolly; some spores produced.
Plates 21 to 24. Gray felt-like growth covering the medium; no spore production.
Plates 25 to 28. These plates differed from plates 21, 22, 23, and 24 in that some of the plates were zoned and produced more spores.

It is clear that there exist variations in a single strain which can not be accounted for on any other ground than the effect of environment. If, therefore, the differences in environment have caused these variations in one year, there may be a possibility of certain environments causing still greater variations which would be more or less permanent.

EFFECT OF THE MEDIUM ON SPORE SIZE

Spores were also measured from the different media to ascertain whether the spore size had been affected. One hundred spores were measured from five different media, and the mean length, mean breadth, standard deviation, and probable error of the mean were calculated for five strains (see Table V). It will be seen that the various media did affect the spore size, but all strains were not affected alike by the same medium. While it has been definitely shown that there exist different strains in *Colletotrichum gloeosporioides*, it also has been shown that these strains are affected in growth characteristics and morphological characters by the medium.

MUTATIONS

The variations which have been described in this paper occurring in the various strains of *Colletotrichum gloeosporioides* have been shown to be due to environmental factors. Not all the variations, however, which occurred during the progress of the work are thought to be due to the environment. These variations which were thought to be induced by some factor or factors other than the environment are in this paper called mutations. These mutations have kept their peculiar characteristics although grown under the same conditions as the cultures from which they arose.

When the various strains were isolated in the fall of 1916, they were grown in plate cultures to study their growth characteristics. The

cultures in which the mutations occurred had greenish gray, fluffy, aerial growth. None of the cultures showed any variation from the description given in the table. This seems to indicate that the cultures were all pure.

TABLE V.—*Differences in spore size of Colletotrichum gloeosporioides induced by various media*

Kind of medium.	Mean spore length in microns.	σ	Mean spore width in microns.	σ
STRAIN 295				
Corn meal agar	11.54 ±0.065	0.97±0.046	5.52±0.057	0.85 ±0.041
Green bean plug	14.13 ± .114	1.69± .081	4.41± .11	1.63 ± .078
Potato agar	13.6 ± .129	1.92± .092	4.9 ± .055	.835± .040
Lactose agar	15.74 ± .319	4.74± .226	4.65± .044	.65 ± .031
Oat agar	13.24 ± .071	1.06± .051	5.34± .018	.27 ± .013
STRAIN 296				
Corn meal agar	9.14 ±0.111	1.65±0.079	5.48±0.052	0.77 ±0.037
Green bean plug	12.01 ± .115	1.71± .082	4.2 ± .065	.97 ± .046
Potato agar	11.87 ± .118	1.75± .083	5.3 ± .03	.44 ± .021
Lactose agar	13.53 ± .103	1.53± .073	4.48± .04	.61 ± .029
Oat agar	11.98 ± .078	1.16± .055	5.23± .127	1.88 ± .090
STRAIN 298				
Corn meal agar	11.036±0.176	2.61±0.124	3.34±0.056	0.836±0.040
Green bean plug	14.79 ± .094	1.40± .067	4.68± .014	.21 ± .010
Potato agar	12.96 ± .144	2.14± .102	4.56± .0188	.28 ± .013
Lactose agar	13.17 ± .126	1.88± .090	4.54± .061	.91 ± .043
Oat agar	12.98 ± .078	1.16± .055	5.56± .064	.95 ± .045
STRAIN 429				
Corn meal agar	13.03 ±0.138	2.05±0.098	3.94±0.036	0.53 ±0.025
Green bean plug	14.75 ± .095	1.42± .068	3.26± .077	1.15 ± .055
Potato agar	13.07 ± .125	1.87± .089	3.99± .122	1.81 ± .086
Lactose agar	12.75 ± .101	1.52± .072	3.75± .047	.70 ± .033
Oat agar	13.76 ± .123	1.81± .086	4.58± .121	1.80 ± .086
STRAIN 651				
Corn meal agar	14.43 ±0.115	1.71±0.082	4.49±0.013	0.19 ±0.009
Green bean plug	17.23 ± .110	1.64± .078	4.52± .035	.52 ± .025
Potato agar	15.11 ± .115	1.7 ± .081	5.12± .017	.25 ± .012
Lactose agar	15.67 ± .121	1.8 ± .086	4.44± .042	.62 ± .030
Oat agar	15.06 ± .113	1.67± .080	5.38± .028	.42 ± .020

In the fall of 1917, after the strains had been grown on artificial media for a year, they were again grown in plate cultures. In a few of the strains there appeared some mycelial growth which differed in color from

the rest of the growth in that plate. These mutations usually appeared as wedge-shaped or fanlike areas with the point of origin usually at the center of the culture. Sometimes they occurred more toward the periphery of the culture. (Pl. 86, A, B.)

Mutations occurred in the following strains: 943, 297, 615, 495, 940, 510, 561, 536, 527, and 990. These mutations have remained true to the characteristics manifested by the first culture. Figure 2 will serve to illustrate the manner in which the mutations originated. Since these strains were not progenies from a single spore, it was thought that there might be a possibility of having a mixture of strains.

There are several types of these variations. One type had a white, fluffy mycelium. A second type, where the mycelium was confined in the medium, had abundant spore production on the surface. A third type had varying shades of gray mycelium bearing spores. At first these peculiarities in growth were regarded as modifications due to some environmental factor. However, after these variations were transferred to other culture tubes and the resulting cultures always exhibited the same characteristics, they then were considered as mutations. Therefore, single-spored cultures were made from one of the strains.

SINGLE-SPORED ISOLATIONS

On November 14, 1917, single spores were isolated from culture 990. The spores were taken from oatmeal agar, and a suspension was made in sterilized distilled water. A platinum loop was used to transfer a drop of the suspension to a cover glass. Each cover glass was examined with the microscope, and when a drop contained only one spore the cover glass was dropped into a test tube containing potato agar. Three cultures were thus obtained and were designated as 990A, 990B, and 990C. After the spores had germinated and had produced a mycelium, transfers were made to the five media used in culturing the various strains. The growth characteristics of cultures 990A, 990B, and 990C were identical with those of the original culture 990.

On November 26, 1917, transfers were made from culture 990C to potato agar plates. The resulting growth was composed of black and white mycelia, with abundant production of spores in the center of the culture (Pl. 86,C). On December 12, 1917, transfers were made from the white and black mycelia to potato agar plates from the cultures made November 26, 1917. The plates made from the black mycelium became black with some white mycelium. The plates made from the white mycelium were white, but only slight traces of black growth could be detected. All cultures produced abundant spores.

On January 9, 1918, transfers were again made from the two kinds of cultures obtained in transfers of December 12, 1917, with results similar to the transfers of December 12, with the exception that there was

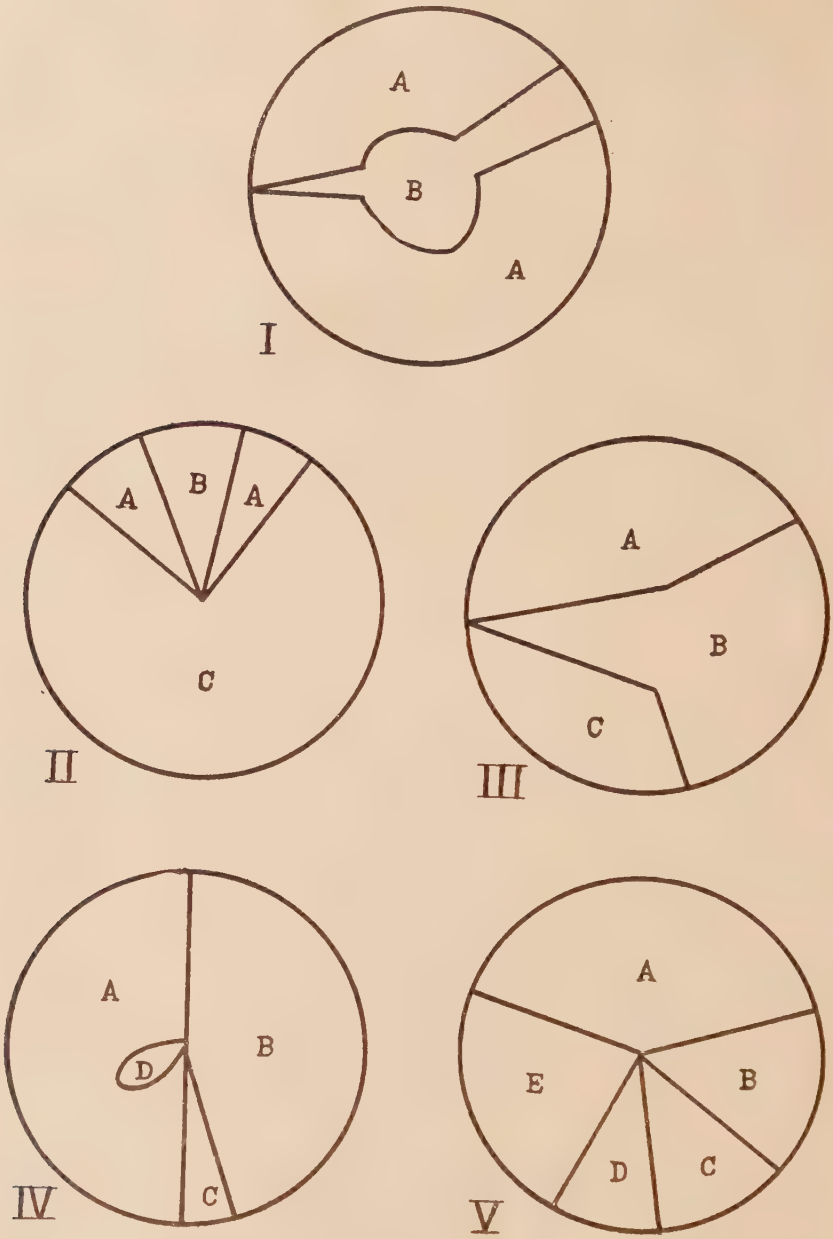


FIG. 2.—I, culture 510: A, greenish black mycelium; B, white mycelium. II, culture 943: A, black mycelium; B, white mycelium; C, mycelium mostly in medium, growth zoned, abundant spore production. III, culture 495: A, black mycelium; B, gray mycelium; C, white mycelium. IV, culture 527: A, gray mycelium; B, greenish black mycelium; C, white mycelium; D, black mycelium. V, culture 940: A, greenish black mycelium; B, white mycelium, some greenish concentric circles; C, black mycelium; D, white mycelium; E, white and black mixed.

practically no black mycelium in the white cultures and but very little white growth in the dark cultures.

Another set of transfers was made on January 24, 1918, from the cultures made January 9, with the result that the white cultures were pure white but the black cultures still produced white hyphae. All plates produced an abundance of spores.

Since the spores are asexual, I wished to determine if they would act like parts of the mycelium when transferred. On January 29, 1918, transfers were made from the spores produced by the white mycelium, and the resulting cultures were pure white, producing many spores. Also spores were transferred from the black and white plates, and the resulting cultures were black with some white hyphae, each culture producing many spores.

The foregoing experiment seems to point to the fact that asexual spores of *Colletotrichum gloeosporioides* act like mycelium when transferred.

The various types obtained by the mutations (fig. 2) are similar to the strains I had in culture. Therefore, one might be led to conclude from the foregoing data that *Colletotrichum gloeosporioides* is constantly giving off new types under natural conditions, as well as in artificial cultures.

SUMMARY

(1) *Colletotrichum gloeosporioides* is a polymorphic species made up of a number of strains.

(2) The various strains when grown on artificial media give distinct cultural characteristics.

(3) Each strain is affected by its environment. The growth characteristics as well as the spore size are varied by the medium on which the strain is grown.

(4) This induced variation may be more or less permanent.

(5) There occur mutations in culture which resemble the strains isolated from the natural environment.

LITERATURE CITED

(1) CLAUSEN, Roy E.
1912. A NEW FUNGUS CONCERNED IN WITHER TIP OF VARIETIES OF CITRUS MEDICA. *In* Phytopathology, v. 2, no. 6, p. 217–235, 1 fig., pl. 21–22. Index to literature, p. 233–234.

(2) EDGERTON, Claude Wilbur.
1908. THE PHYSIOLOGY AND DEVELOPMENT OF SOME ANTHRACNOSES. *In* Bot. Gaz., v. 45, no. 6, p. 367–408, 17 fig., pl. 21. Literature cited, p. 405–407.

(3) ESSIG, E. Ó.
1911. WITHER-TIP OF CITRUS TREES (COLLETOTRICHUM GLOEOSPOROIDES Penzig). *In* Pomona Col. Jour. Econ. Bot., v. 1, no. 1, p. 25–56, fig. 14–21.

(4) FAWCETT, Howard S.
1915. CITRUS DISEASES OF FLORIDA AND CUBA COMPARED WITH THOSE OF CALIFORNIA. *In* Cal. Agr. Exp. Sa. Bul. 262, p. 149–211, 24 fig.

(5) Rolfs, P. H.
1904. wither-tip, and other diseases of citrus trees and fruits caused by colletotrichum gloeosporoides. U. S. Dept. Agr. Bur. Plant Indus. Bul. 52, 20 p., 6 pl.

(6) Shear, C. L., and Wood, Anna K.
1913. studies of fungous parasites belonging to the genus glomerella. U. S. Dept. Agr. Bul. 252, 110 p., illus., 18 pl. on 9 l. Literature cited, p. 101–105.

(7) Stoneman, Bertha.
1898. a comparative study of the development of some anthracnoses. *In* Bot. Gaz., v. 26, no. 2, p. 69–120, pl. 7–18. Bibliography, p. 114–117.

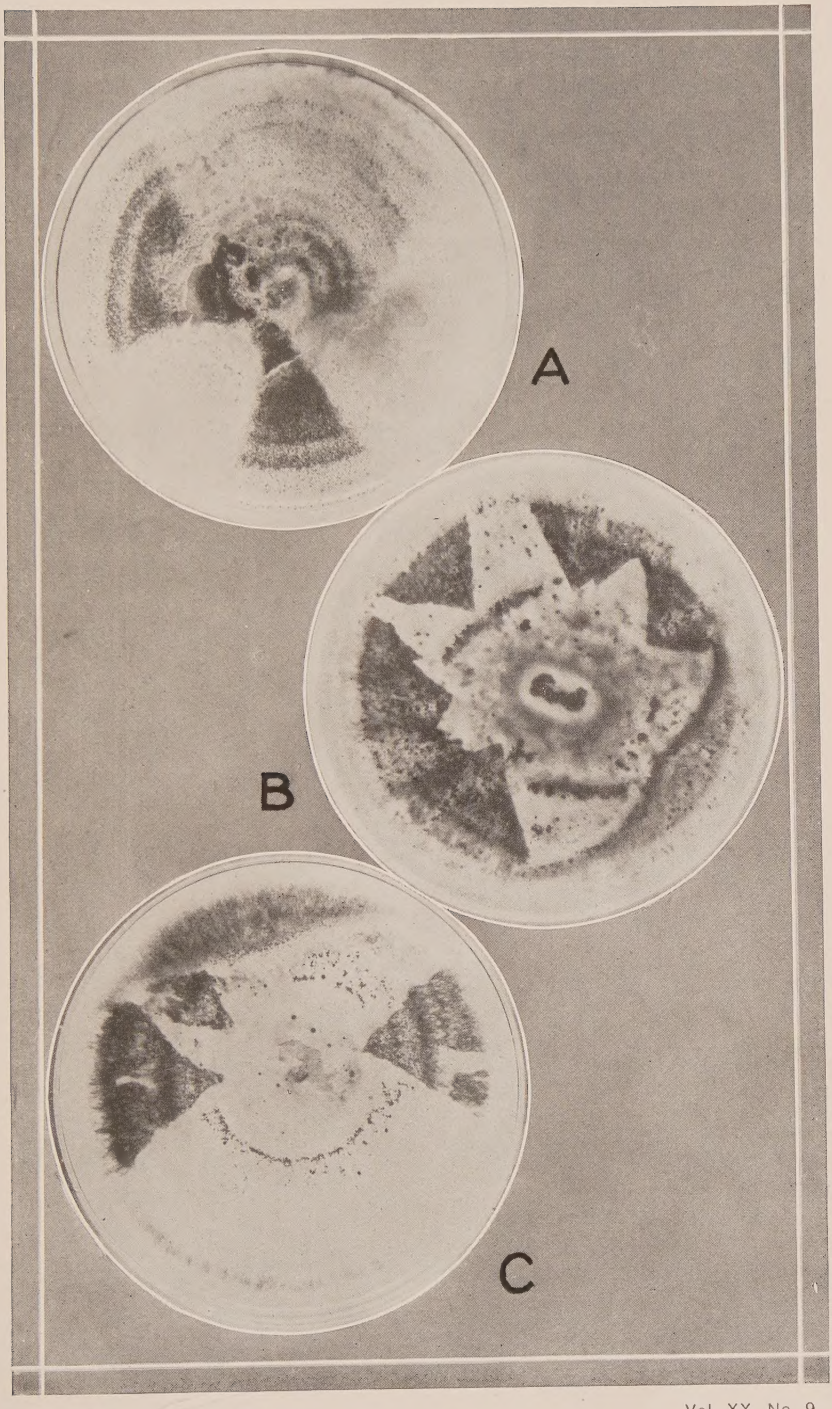
(8) Underwood, Lucien M.
1891. diseases of the orange in florida. *In* Jour. Mycol., v. 7, no. 2, p. 27–36.

(9) U. S. Department of Agriculture. Bureau of Plant Industry.
1908. report of the chief of the bureau of plant industry, 1907. 93 p. Washington, D. C.

PLATE 86

A, B.—Variation occurring in strain 990. The cultures were not made from a single spore.

C.—Variation occurring in a culture of strain 990 which was made from a single spore.


A
B
C